varieties (Cosgrove et al., 2004), the histone variants H3.3 and H2A.Z (Jin and Felsenfeld, 2007), and chromatin-associated proteins that bind to modified histones or to methylated DNA (Li et al., 2007). These effectors of nucleosome stability have often been referred to as "marks," leaving open the question of how marking can result in gene activation or repression and epigenetic maintenance of these states. But if these diverse chromatin modifiers act by simply increasing or decreasing nucleosome stability, then we may be much closer to a precise molecular understanding of epigenetic inheritance in development and disease.

REFERENCES

Boeger, H., Griesenbeck, J., Strattan, J.S., and Kornberg, R.D. (2003). Mol. Cell *11*, 1587–1598.

Cosgrove, M.S., Boeke, J.D., and Wolberger, C. (2004). Nat. Struct. Mol. Biol. *11*, 1037–1043.

Fatemi, M., Pao, M.M., Jeong, S., Gal-Yam, E.N., Egger, G., Weisenberger, D.J., and Jones, P.A. (2005). Nucleic Acids Res. *33*, e176.

Jin, C., and Felsenfeld, G. (2007). Genes Dev. *21*, 1519–1529.

Kladde, M.P., Xu, M., and Simpson, R.T. (1996). EMBO J. *15*, 6290–6300.

Li, B., Carey, M., and Workman, J.L. (2007). Cell *128*, 707–719.

Lin, J.C., Jeong, S., Liang, G., Takai, D., Fatemi, M., Tsai, Y.C., Egger, G., Gal-Yam, E.N., and Jones, P.A. (2007). Cancer Cell, this issue.

Mito, Y., Henikoff, J., and Henikoff, S. (2005). Nat. Genet. *37*, 1090–1097.

Reinke, H., and Horz, W. (2003). Mol. Cell *11*, 1599–1607.

van Leeuwen, F., and van Steensel, B. (2005). Genome Biol. *6*, 113.

In Situ Carcinoma—Can We Predict which Patient Will Come Back with a Recurrence?

Peter T. Simpson,[1,2] Leonard M. Da Silva,[1,2] and Sunil R. Lakhani[1,2,3,*]
[1]Molecular and Cellular Pathology, School of Medicine, University of Queensland, Herston QLD 4006, Australia
[2]Queensland Institute of Medical Research, Herston QLD 4006, Australia
[3]Pathology Queensland, The Royal Brisbane and Women's Hospital, Brisbane QLD 4029, Australia
*Correspondence: s.lakhani@uq.edu.au
DOI 10.1016/j.ccr.2007.10.026

The frequency of in situ carcinomas has been rising since the introduction of mammographic screening. The management of patients with preinvasive disease remains difficult due to our lack of ability to accurately predict which patients will recur and progress to invasive carcinoma. Although some factors, such as lesion size and extent of margin clearance, are strong predictors of recurrence, many patients are still under- or overtreated. In this issue of Cancer Cell, Gauthier and colleagues suggest that abrogated response to cell stress measured by analysis of p16 and the proliferation marker Ki67 accurately predicts recurrence in ductal carcinoma in situ.

Ductal carcinoma in situ (DCIS) is a heterogeneous disease, diagnosed with increasing frequency since the introduction of the mammographic screening program (Hofvind et al., 2007). A number of classification systems have been proposed, primarily based on nuclear morphology. The type of DCIS, lesion size, and most importantly, distance to excision margin have been shown to be strong predictors of recurrence (MacDonald et al., 2005). Approximately half of recurrences remain in situ, while half will be invasive. Patients with invasive carcinoma are at risk of metastases, and hence this represents a significant event for the patient.

Several studies have demonstrated that the risk of in situ and invasive recurrence is greater for high-grade compared to low-grade DCIS. This would suggest that more aggressive therapy is indicated for high-grade lesions, and although the best treatment for DCIS is still uncertain, there is little doubt that a margin ≥10 mm, endocrine therapy, and radiotherapy following excision reduces the risk of recurrence (MacDonald et al., 2005; Burstein et al.,2004). Nonetheless, it is clear that our ability to accurately predict which patient will recur is limited, leading to under- or overtreatment.

In the current issue of *Cancer Cell*, Gauthier et al. (2007) suggest that a simple panel of markers may solve that problem. DCIS with high P16+ and/or COX2+ and high Ki67+ confers a significant risk of subsequent in situ and invasive recurrence. What

is the basis for this, and should we all be rushing to add this panel to our routine practice?

Three factors are thought to be important in the development of cellular senescence: DNA damage, telomere shortening, and suppression of the *INK4a/ARF* locus (Collado et al., 2007). Collectively, these mechanisms protect from uncontrolled proliferation and hence tumor formation. The authors argue that mechanisms that lead to abrogation of such a pathway would contribute to tumorigenesis and may help to predict DCIS that is likely to progress. They studied the expression of p16 (INK4a) in a cohort of 70 DCIS, 38 that did not progress and 32 with recurrent DCIS or invasive carcinoma. Eighteen of seventy DCIS lesions had high p16+ (≥25% cells), and this was independent of grade and ER (estrogen) status. Interestingly, the p16 expression did not predict for subsequent behavior.

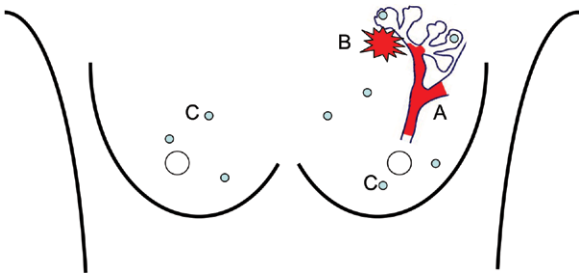

Figure 1. Tumor Recurrence: DCIS versus LCIS
Ductal carcinoma in situ (DCIS) (A) is mostly a segmental disease and can be removed by local excision. Risk factors for recurrence include grade, size, and margin of excision. The paper by Gauthier et al. suggests that high p16+ and/or high COX2 and high Ki67+ predict for in situ and invasive recurrence (B). In contrast, lobular carcinoma in situ (LCIS) (C) is a multifocal and in some patients a bilateral disease and hence cannot be removed by segmental excision. Although the risk of recurrence is skewed to the ipsilateral breast, some women will get invasive cancer in the opposite breast; hence, management is even more problematic.

p16 overexpression can occur under two different circumstances, either as a result of cellular stress on a background of a functional p16/RB pathway leading to senescence or on a background of suppressed or compromised RB pathway, where it is overexpressed due to loss of negative feedback. Hence, despite the same end result (P16+), the effect on cell proliferation would be quite different. Staining for Ki67, a proliferation marker, demonstrated 26/70 DCIS with a high Ki67 index (>10%). Of the 18 cases with high p16+, 8 also had high Ki67+, and all had subsequent recurrence, 5 of these with invasive disease. Of the ten p16+ DCIS with low Ki67+, only one DCIS developed a recurrence. Hence, DCIS that progress appear to have a compromised RB pathway.

A number of groups have demonstrated that the pathological grade and genetic changes in the recurrence mirror the primary DCIS (Millis et al., 2004). The authors hypothesized that the high p16+/Ki67+ seen in DCIS would therefore also be a hallmark of invasive carcinoma. In a set of 130 invasive cancers analyzed for expression profiling, the authors found an association between p16 mRNA expression and the "basal-like" breast cancers. They also demonstrated that these basal cancers had low levels of RB, high E2F3, high Cyclin E, and low Cyclin D. Further, the authors noted that COX2 overexpression was also a feature of basal cancers. Interestingly, like p16, COX2 expression in the DCIS cohort did not predict for recurrence on its own but was predictive if combined with Ki67 status. The authors went on to demonstrate that overexpression of COX2 in high p16+/Ki67+ DCIS was a consequence of the deregulated p16/RB pathway.

Hence, the authors conclude that high p16+ and/or COX2+ and Ki67+ predict for recurrence and that this phenotype is that of invasive basal cancers. Although the numbers of cases studied is very small for subset analysis, the authors make the point that 6/26 high-grade DCIS had a high p16+/Ki67+ phenotype, and all developed recurrences, suggesting that this panel may also be able to stratify high-grade lesions into clinically meaningful groups.

In a further twist to the story, the authors report that half their cases of DCIS with a high p16+ and/or COX2+ and Ki67+ were positive for ER, despite the finding that this phenotype is associated with a basal invasive cancer, a tumor type that is often though not always ER negative. Since the "triple-positive" DCIS in their hands is invariably linked to a subsequent recurrence (often invasive), the authors argue that basal like tumor may arise from both ER+ and ER− DCIS. There is evidence in the literature, although little highlighted, that basal cancers are heterogeneous with relation to prognosis and are not universally "bad" (Fulford et al., 2007). Perhaps here we have a hint regarding the biology underlying this heterogeneity. The rush to assign histogenetic relationships ("basal cancers arise from basal cells") may also be too simplistic, as it does not take into account cellular plasticity (Gusterson et al., 2005).

A larger series may help in refining the role of ER in the pathogenesis of DCIS and its progression to invasive cancer. The hypothesis that ER+ DCIS with an abrogated response may be "dead-end" lesions is unlikely, as there is compelling evidence that the stroma may play a significant role in disease progression (Orimo et al., 2005) and that, given the same starting cell population, the conditions in which the cells grow can substantially alter the type of tumor that develops (Ince et al., 2007).

So should we incorporate this "triple test" into our routine clinical practice? Clearly the study is tantalizing but at this stage very small and needs validating in a larger set as well as in prospective studies. In this small cohort it appears to be highly predictive; however, the panel does not identify all patients that recurred, and hence the true sensitivity and specificity of the test are unclear.

It is implicit but not always intuitive that the lesion studied is not the one that progresses, since it is out of the patient. The role of the biological pathway in any lesion of equivalent grade or

worse left behind, or in normal tissues, remains unclear. Further, the study does not address the issue of heterogeneity within the same sample.

There is also a need to correlate the findings with clinicopathological features and to be able to carry out subset analysis, in particular expanding the analysis of the high-grade/high-p16/high-Ki67 DCIS. This issue also highlights very clearly that a combination of pathological, clinical, and molecular factors may ultimately reveal more powerful and robust measures for disease classification than any one modality alone (Rosai, 2007).

The ability to predict the outcome of an in situ cancer at the time of primary diagnosis would make a huge impact in clinical practice, especially as the frequency of the lesions is rising due to mammographic screening. The authors have set the stage for DCIS, which is generally a segmental disease (Figure 1). Perhaps this will also be the spur to study lobular carcinoma in situ (LCIS), a multifocal proliferation with a bilateral risk of invasive carcinoma, and hence an even bigger dilemma regarding appropriate management.

REFERENCES

Burstein, H.J., Polyak, K., Wong, J.S., Lester, S.C., and Kaelin, C.M. (2004). N. Engl. J. Med. *350*, 1430–1431.

Collado, M., Blasco, M.A., and Serrano, M. (2007). Cell *130*, 223–233.

Fulford, L.G., Reis-Filho, J.S., Ryder, K., Jones, C., Gillett, C.E., Hanby, A., Easton, D., and Lakhani, S.R. (2007). Breast Cancer Res. *9*, R4.

Gauthier, M.L., Berman, H.K., Miller, C., Kozakeiwicz, K., Chew, K., Moore, D., Rabban, J., Chen, Y.Y., Kerlikowske, K., and Tlsty, T.D. (2007). Cancer Cell, this issue.

Gusterson, B.A., Ross, D.T., Heath, V.J., and Stein, T. (2005). Breast Cancer Res. *7*, 143–148.

Hofvind, S., Sorum, R., and Thoresen, S. (2007). Acta Oncol., 1–7. Published online September 12, 2007.

Ince, T.A., Richardson, A.L., Bell, G.W., Saitoh, M., Godar, S., Karnoub, A.E., Iglehart, J.D., and Weinberg, R.A. (2007). Cancer Cell *12*, 160–170.

MacDonald, H.R., Silverstein, M.J., Mabry, H., Moorthy, B., Ye, W., Epstein, M.S., Holmes, D., Silberman, H., and Lagios, M. (2005). Am. J. Surg. *190*, 521–525.

Millis, R.R., Pinder, S.E., Ryder, K., Howitt, R., and Lakhani, S.R. (2004). Br. J. Cancer *90*, 1538–1542.

Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V.J., Richardson, A.L., and Weinberg, R.A. (2005). Cell *121*, 335–348.

Rosai, J. (2007). Lab. Invest. *87*, 403–408.

NOTCH and PI3K-AKT Pathways Intertwined

Alejandro Gutierrez[1,2] and A. Thomas Look[1,2,*]
[1]Department of Pediatric Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[2]Division of Hematology/Oncology, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115, USA
*Correspondence: thomas_look@dfci.harvard.edu
DOI 10.1016/j.ccr.2007.10.027

Constitutive signaling by the NOTCH1 receptor contributes to more than half of all cases of T cell acute lymphoblastic leukemia (T-ALL). However, blocking the proteolytic activation of NOTCH1 with γ-secretase inhibitors (GSIs) fails to alter the growth of some T-ALL cell lines carrying the mutated receptor. A recent report by Palomero et al. in *Nature Medicine* identifies loss of PTEN as a critical event leading to resistance to NOTCH inhibition, which causes the transfer of "oncogene addiction" from the NOTCH1 to the PI3K/AKT pathway. This novel observation suggests the need to simultaneously inhibit both pathways as a means to improve therapeutic efficacy in human T-ALL.

NOTCH1 encodes a transmembrane receptor that undergoes a series of activation steps upon ligand binding, culminating in the γ-secretase-mediated proteolytic release of the intracellular fragment of NOTCH1 (ICN). The ICN then translocates to the nucleus, where it is transcriptionally active and required for T cell development (reviewed in Grabher et al., 2006). Aberrant NOTCH1 activation leads to T-ALL in the mouse, and activating mutations occur in more than 50% of cases of human T-ALL (Weng et al., 2004). GSIs, which were initially developed for the treatment of Alzheimer's disease, effectively inhibit the proteolytic activation of NOTCH receptors, a discovery that led to enthusiastic application of NOTCH pathway inhibitors to block cell proliferation and survival in T-ALL. Inhibition of NOTCH1 signaling by GSI treatment proved effective in inducing proliferation arrest or apoptosis in some but not all T-ALL cell lines, suggesting a previously unrecognized mechanism of resistance. In a recent report in *Nature Medicine*, Palomero et al. (2007) show that homozygous loss of *PTEN* is a critical determinant of resistance to GSI-mediated inhibition of NOTCH1 signaling in T-ALL cell lines (Figure 1). They show further that *PTEN* expression is negatively regulated by HES1, a prominent